REMARKS

The present application is directed to compositions for the inhibition of cellular proliferation by the administration of Tissue Factor Pathway Inhibitor (TFPI), TFPI homologs, or active fragments thereof. The TFPI, TFPI homologs, or active fragments thereof, are combined with a pharmaceutically acceptable excipient and are useful for treating diseases associated with undesirable cell proliferation including angiogenesis and angiogenesis-related diseases. Claims 11, 12, 14-18 and 20 are pending in the above-identified patent application.

Claim Rejections 35 U.S.C. §112, first paragraph

Claims 12 and 14-17 remained rejected under 35 U.S.C. §112, first paragraph, for lack of enablement for the same reasons as set forth in April 22, 2003 Office Action. The Office Action asserted, "the specification, while being enabling for 'active' fragments comprising Kunitz-3 domain, does not reasonably provide enablement for any 'active fragments of Kunitz-3 domain.'" Applicants respectfully traverse the rejection.

Applicants assert that the specification is enabling for any active fragment of the Kuinitz-3 domain. As previously submitted, the specification states on page 5, lines 1-6, that "[t]he composition provided herein contain[s] a protein known as "tissue factor pathway inhibitor" (TFPI), a TFPI homolog, or an active fragment thereof, wherein the fragment is defined by its ability to exhibit antiproliferative activity on human and other animal endothelial cells." The specification additionally states on page 7, lines 17-19, that "[a]ctive fragments of TFPI are further defined herein as fragments having an inhibitory or repressive effect on angiogenesis," and, further, on page 19, line 30, through page 20, line 2, that "Kunitz-3 or an active portion thereof most probably plays an important role affecting the activity of TFPI or the binding of TFPI to its receptor on the surface of the endothelial cells" (emphasis added). On pages 11 and 12 (section *TFPI Fragments*) the specification teaches how to produce TFPI fragments and test them for antiproliferative activity.

claimed.

Claim Rejections under 35 U.S.C. §102(a) and §103(a)

Claims 11-17 and 19 remained rejected under 35 U.S.C. §102(a) as anticipated by Steinhubl *et al.* (J. Amer. College of Cardiology 29 (2), 243A, 1997) or Khouri *et al.* (Surgical Forum 46, 389-391, 1995) for the same reasons as set forth in the April 22, 2003 Office Action. The Office Action stated "[I]f the prior art teaches the identical chemical structure and composition, the properties applicant discloses and/or claims are necessarily present." Applicants respectfully traverse this rejection because the prior art does not teach the identical chemical structure and composition. Claim 20 remained rejected under 35 U.S.C. §103(a) as obvious over Steinhubl *et al.* or Khouri *et al.* for the same reasons as set forth in the April 22, 2003 Office Action.

As previously submitted Steinhubl *et al.* and Khouri *et al.* teach the inhibition of neointimal proliferation by a whole TFPI molecule. In contrast Claim 11 recites a Kunitz-3 domain fragment of a tissue factor pathway inhibitor. Therefore, what is presently claimed is not identical to what is taught by the prior art.

Applicants respectfully assert the compositions of the present invention as recited in Claims 11-17 and 19, are not *prima facie* anticipated by or obvious over Steinhubl *et al.* or Khouri *et al.*

The Examiner cited Khouri *et al.* and Steinhubl to demonstrate use of TFPI for inhibiting neointimal hyperplasia resulting from blood vessel damage. As discussed in applicants' previous response, neointimal hyperplasia is unrelated to endothelial cell proliferation. Intimal hyperplasia is defined by the abstract of Davies *et al.*[1] as the "abnormal migration and proliferation of vascular smooth muscle cells with the associated deposition of extracellular connective tissue matrix"[2]. Neointimal hyperplasia, therefore, is characterized by uncontrolled proliferation of smooth muscle cells and not endothelial cells.

Neointimal hyperplasia is related to blood vessel damage, and accordingly some endothelial cell proliferation may be observed. However, neointimal hyperplasia is not an

angiogenesis-related disease, but is a result of an aberration in smooth muscle proliferation. In contrast to the role of smooth muscle cells in neointimal hyperplasia, proliferation of endothelial cells may actually inhibit unregulated smooth muscle cell replication and suppress neointimal hyperplasia. For example, Zagre *et al.*[3] have used growth factors and heparin to stimulate endothelial cell proliferation and to inhibit smooth muscle cell *in vitro* and *in vivo*. Applicants maintain, however, that although endothelial and smooth muscle cells are involved in a response to blood vessels damage both cells are distinctively different with respect to function, morphology, expression of specific markers and proliferation. A few of the major differences between endothelial and smooth muscle cells are summarized in Table 1 below.

TABLE 1

CELL TYPE	ORIGIN	SYSTEM	FUNCTION	SPECIFIC MARKERS
Endothelial	Mesoderm	Vascular	Lining of blood vessel.	CD31 (or PECAM), von Willenbrand, H antigen.
Smooth Muscle	Mesoderm	Muscle	Involuntary muscle contraction.	Smooth muscle actin.

In addition, based on the level of knowledge by one skilled in the art, one would not conclude that endothelial and smooth muscle cells are functionally equivalent or that their proliferation can be triggered or inhibited simultaneously by same agents. Although there are some agents that can affect both endothelial and smooth muscle cells in the same way, there are numerous teachings in the art that teach the opposite: that one agent can affect endothelial and smooth muscle cells in different, and many times opposite ways. Examples of such teachings are provided below in Table 2:

[3] Zagre et al., "Local delivery of [illegible] reduces intimal hyperplasia in a canine carotid artery balloon injury model," *J. Vasc. Surg.* 25:840-848 (1997).

TABLE 2

Agent/Signal	Effect on Smooth Muscle Cells	Effect on Endothelial Cells
Insulin	Stimulates Proliferation Ridray S., Hyperinulinemia and smooth muscle proliferation. *Int. J. Metab. Disord* 19: S39-51 (1995)	No Effect On Proliferation Nakao-Hayashi, J. *et al.* Stimulatory effect of insulin and insulin-like growth factor I on migration and tube formation by vascular endothelial cells. *Atherosclerosis* 92:141-149 (1992)
Alpha tocopherol	Inhibits Proliferation Devaraj S. *et al.* The effects of alpha tocopherol on critical cells in atherogenesis. *Curr. Opin. Lipidol.* 9:11-5 (1998)	Stimulates Proliferation Kuzuya *et al.* Antioxidants stimulate endothelial cell proliferation in culture. *Artery* 18:115-120 (1991)
Vanadate	Induces Cell Death Daum *et al.* The mitogenic activated protein kinase pathway contributes to vanadate toxicity in vascular smooth muscle cells. *Mol. Cell Biochem.* 183:97-103 (1998)	Stimulates Proliferation Maher, P.A. Stimulation of endothelial cell proliferation by vanadate is specific for microvascular endothelial cells. *J. Cell Physiol.* 151:549-554 (1992)
Secretoneurin	Stimulates Proliferation Kahler *et al.* Response to vascular smooth muscle cells to neuropeptide secretoneurin. A functional role for migration and proliferation *in vitro*. *Arterioscler. Thromb.* 17:2029-2035 (1997)	Inhibits Proliferation Kahler *et al.* Inhibition of proliferation and stimulation of migration of endothelial cells by secretoneurin *in vitro*. *Arterioscler. Thromb. Vasc. Biol.* 17:932-939 (1997)
Tamoxifen	No Effect Somjen *et al.* effects of gonadal steroids and their antagonist on DNA synthesis in	Inhibition of Proliferation Somjen *et al.* effects of gonadal steroids and their antagonist on

In addition to the different effects of various agents and signals on smooth muscle cells and endothelial cells, both types of cells also respond differently to various mitogenic and inhibitory signals. For example, in smooth muscle cells, 17β-estradiol E_2 and dihydrotestosterone (DHT) have a biphasic effect: low concentrations *stimulate* proliferation, while high concentrations *inhibit* proliferation. In endothelial cells, E_2 and DHT *promote* proliferation in a dose-dependent manner. In smooth muscle cells, E_2 and DHT *inhibit* platelet-derived growth factor (PDGF) or insulin-like growth factor (IGF-1) induced proliferation, while they *enhance* PDGF or IGF-1 driven growth of endothelial cells.[4]

In light of the above discussion, and examples of scientific studies demonstrating the contrasting effects of various signals and factors on smooth muscle cells and endothelial cells, applicants assert that it would not have been obvious to predict the effect of a fragment of TFPI, namely the Kunitz-3 domain, on endothelial cells based on the effect of TFPI on smooth muscle cells. As demonstrated in Table 2, current state-of-the-art scientific research teaches that one skilled in the art could not predict the response of smooth muscle cells to a particular signal based on the response of endothelial cells (and vice versa). Different cell types respond differently to various signals, and such responses are typically the result of the presence or absence of other signals in the immediate environment as well as the presence or absence of specific receptors on the cell surface. Even though in some instances both endothelial and smooth muscle cells respond similarly to a particular signal, applicants have provided at least five examples when cell response is in fact different, if not opposite.

Given the lack of predictability with regard to cell response to various signals, applicants respectfully submit that the cited references pertaining to the use of TFPI for inhibiting neointimal hyperplasia, do not anticipate or render the present invention obvious. Applicants' invention is directed to compositions comprising the Kunitz-3 domain of TFPI for inhibiting proliferation of endothelial cells. Based on the above discussion along with the scientific references provided in Table 2, one skilled in the art could not predict the effect of TFPI on endothelial cells based on the effect of TFPI on smooth muscle cells. Accordingly applicants request that the Examiner withdraw this rejection.

Applicants respectfully submit that the foregoing remarks sufficiently demonstrate that neither Steinhubl et al. or Khouri et al. either anticipate or render the present

invention obvious. Reconsideration and withdrawal of the rejections is therefore respectfully requested.

The foregoing is submitted as a full and complete response to the Advisory Action mailed April 22, 2003. Applicants respectfully submit that the claims are fully enabled, novel and non-obvious over the cited art. Applicants assert that the claims are now in condition for allowance and respectfully request that the application be passed to issuance. If the Examiner believes that any informalities remain in the case, which may be corrected by Examiner's amendment, or that there are any other issues which can be resolved by a telephone interview, a telephone call to the undersigned attorney at (404) 815-6500 is respectfully solicited.

Respectfully submitted,

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